OXYGEN REQUIREMENTS OF SOME HAWAIIAN TUNA BAITFISH

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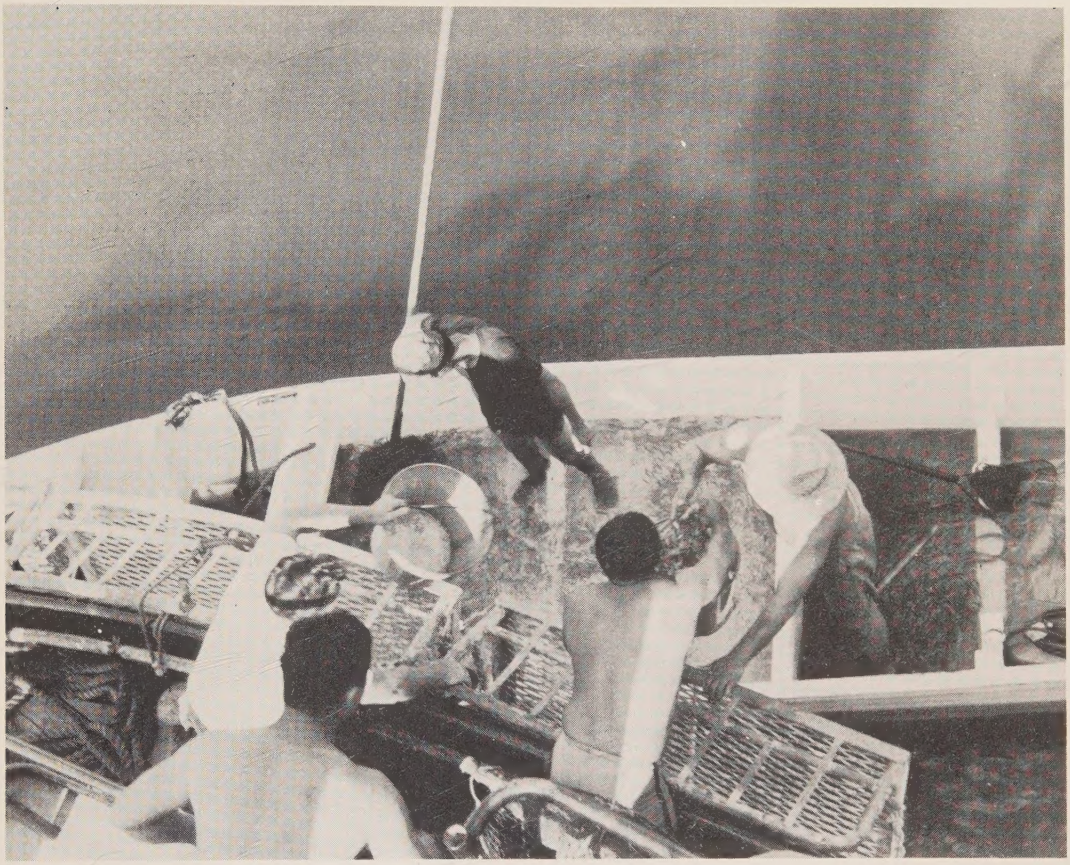

OXYGEN REQUIREMENTS OF SOME HAWAIIAN TUNA BAITFISH

By

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Contribution No. 61 from the Hawaii Marine Laboratory

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The success of livebait fisheries for tuna depends to a large extent on the availability of baitfish. In the Hawaiian fishery for aku (skipjack or striped tuna), Katsuwonus pelamis (Linnaeus), the two species of baitfish most commonly used are the nehu, Stolephorus purpureus Fowler, and the iao, Pranesus insularum (Jordan and Evermann). The nehu, although the less hardy of the two, is the preferred bait. Nehu are generally caught at night by attraction to a light, and both nehu and iao are caught during the day by means of surround nets. Bait is quickly transferred from the nets to livebait wells in the fishing vessels.[1/] The period following the transfer of bait is crucial in livebait fishing, for if water circulation in the live-wells is inadequate and the fish crowd together at the surface or in a corner of the well, considerable mortality may occur. The usual mortality is about 20-30 percent, most of it during the first day, but in some instances it may amount to 50-100 percent.

Heavy bait mortality is probably due to the interaction of several factors. Loss of scales and collisions with the sides of the tank probably lead, directly or indirectly, to the death of many fish. On the other hand, when fish are confined to a limited space, as they are in the livebait wells, the possible depletion of oxygen to levels causing abnormal respiration and suffocation must be considered. This problem of oxygen depletion would be especially acute in those boats (making up the majority of the fleet at present) which employ the method of circulating water through holes in the bottom the well. A few boats possess pumps for circulating water through the live-wells, and may perhaps be less concerned with the problem of oxygen depletion per se. Other questions arise, however, particularly in regard to the effect of different flow rates and various degrees of crowding upon the behavior of the fish. The local annual range of temperature is small, but the possibility of a small but sudden rise or fall in temperature, with its concomitant effects on respiratory metabolism, must be considered.

The object of the present study was to analyze, in the laboratory, the oxygen requirements of the local tuna baitfish in the hope of obtaining basic information which might be of value to livebait fisheries. An investigation of oxygen consumption under various conditions was carried out, emphasis being placed on the effects of temperature, flow rates, various degrees of crowding, and the oxygen concentration of the water. In addition, experiments were designed to determine lethal levels of oxygen for the fish.

The author takes this opportunity to thank Dr. P. B. van Weel for his guidance and constant encouragement throughout this work. Grateful acknowledgement is made to the staff of the Pacific Oceanic Fishery Investigations for advice and technical assistance. Special appreciation is also due Lester Zukeran and Charles Nakamoto of the Hawaii Marine Laboratory for taking a large share of the responsibility for catching and maintaining the stocks.

REVIEW OF THE LITERATURE

An enormous literature exists on respiratory exchange in fish and an extensive review will not be attempted here. A summary of literature relevant to this study will be presented now with mention of individual publications in connection with the appropriate sections of the work. Suehiro's (1951) study of the lethal limits of oxygen for Japanese sardines and anchovies is the only set of observations known to the author on the respiratory metabolism of baitfishes. Besides measuring lethal values of oxygen, Suehiro made several observations on the amount of oxygen consumed by baitfish in live-wells, and emphasized the superior bait-holding capacity of those bait tanks in which water was circulated by pumps.

1/ A good description of the Hawaiian livebait fishery is given by Fred C. June (1951, Commercial Fisheries Review 13(2):1-18).

Note: Financial support for this study came from a U. S. Fish and Wildlife Service fellowship. The material reported forms the greater part of a thesis submitted to the graduate school of the University of Hawaii in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The author at present is a faculty member of the Department of Zoology, Oregon State College, Corvallis, Oregon.

The important factors of temperature and oxygen concentration have received considerable attention from investigators of fish metabolism. At present, the general nature of the effect of temperature and the importance of temperature acclimation seem clearly established (Ege and Krogh 1914; Wells 1935b, c; Sumner and Lanham 1942; Haugaard and Irving 1943), but observed differences among species make it imperative to analyze the influence of temperature on metabolic rate for each species of fish investigated. As regards the effect of oxygen concentration, it would appear that most fish are able to maintain a fairly constant level of metabolism over a wide range in oxygen concentrations (Gaarder 1918; Hall 1929; Keys 1930a, b; Lindroth 1940). There is, however, considerable variation among species as regards the critical concentration, the point at which the rate of oxygen consumption begins to decline. The role of breathing regulation in helping to maintain a constant metabolic rate as the oxygen content falls below normal has been pointed out by several workers (Westerlund 1906; Winterstein 1908; Belding 1929; Meyer 1935; van Dam 1938), but breathing movements and rate of oxygen consumption have rarely been measured at the same time.

The effect of aggregation size on metabolic rate in fish has received comparatively little attention since the initial discovery by Schuett (1933) of a "group effect" in goldfish. Essentially, he found that groups of four fish consumed less oxygen per unit weight than single fish in the same size container. His results received confirmation from Shlaifer (1938) and later Geyer and Mann (1939a, b) reported a "group effect" for the perch (Perca fluviatilis). Large groupings of fish have apparently not been investigated in this regard.

With the notable exception of Hall (1930), almost all the lethal values of oxygen reported in the literature pertain to freshwater fish (Chapman 1939; Moore 1942; Wells 1913; Townsend and Earnest 1940). Individual differences in resistance to low oxygen are pronounced, as indicated by wide range in lethal oxygen values within a species (Wilding 1939). Furthermore, lethal values of oxygen have been shown to vary with temperature and possibly with the size of the fish (Graham 1949; Keys 1931; Moore 1942).

Finally, it is apparent that most of the investigations on fish metabolism in general have been concerned with freshwater species and a need exists for more work on marine forms.

CAPTURE, MAINTENANCE, AND HANDLING OF STOCKS

Practically all the experimental work was performed between 1950 and 1953 at the Hawaii Marine Laboratory, located on Coconut Island in Kaneohe Bay, Oahu. Baitfish, particularly iao, were fairly abundant around the island and could be readily seined in shallow waters. Occasionally fish were attracted to a submerged light and caught in a net lifted from below. Initially stocks were kept in large concrete cisterns some distance away and transferred as needed to aquaria at the laboratory. Later a shallow wooden box (fig. 1) was built for keeping large numbers of fish at the laboratory. Water circulation in this container was efficient and the fish could be easily captured. The fish were fed bread crumbs daily in the early afternoon.

Although both the nehu and iao are delicate fish, the nehu in particular is highly excitable and suffers hemorrhages and loss of scales from rubbing against the sides of the tank. Suehiro (1951) mentions this as one of the principal causes of death of the anchovies (Engraulis japonicus) and sardines (Sardinia melanostica) used for bait in the Japanese tuna fishery. By the end of the summer of 1950 it was obvious that the nehu was unsuitable as an experimental animal and attention henceforth was focused almost entirely on the iao.

Stock mortalities were greatest in the summer months, and large mortalities coincided with severe hot weather, with the water temperature often rising above 29° C. Summer fish, on the whole, behaved more erratically in experiments than winter fish, and seemed particularly susceptible to small increases in temperature. Several summer experiments had to be discarded at the outset because of the obviously abnormal behavior of the fish.

Figure 1.--Holding box for iao at the main laboratory

Two additional factors regarding stock "condition" must be considered. First, fish with injured tails, presumably chewed by other fish, were sometimes seen in the tanks. Their swimming behavior appeared to be normal. Secondly, an ectoparasite was occasionally found attached to the underbelly of the fish. To estimate the incidence of this kind of parasitism, two samples taken near the laboratory were examined. In the first sample, 1 fish out of a total of 95 showed ectoparasites; and in the second sample, only 2 fish out of 390 possessed externally visible parasites. Occasionally, one or two fish with parasites or chewed tails were discovered at the end of an experiment, but the presence of these fish did not appear to change the pattern of the results.

In view of the delicate nature of these fish, considerable care was taken in selecting and handling experimental animals. Fish dissociated from the main school and swimming weakly at the surface were removed as quickly as possible from the stock tanks. In transferring the fish, they were first lifted from the tank in a fine-meshed net, dipped into a beaker, and then poured through a plastic funnel into the experimental container.

MEASUREMENT OF DISSOLVED OXYGEN

The unmodified Winkler procedure was used to measure dissolved oxygen for all the experiments in which the water in the experimental container was continually being renewed. The method is standardized and widely used and, therefore, needs no detailed description. Essentially, the steps outlined by Tully et al. (1950) were followed both in the preparation of reagents and in the collection and preserving of samples. In those experiments where the fish were allowed to use up the dissolved oxygen in sealed jars, the permanganate modification of Winkler's method was employed to avoid possible nitrite error. The procedure followed that outlined by Welch (1948).

Several methods are available for standardizing sodium thiosulfate solutions, and their relative utility has recently been compared (Thompson and Robinson 1939). Potassium bi-iodate, as recommended by Thompson and Robinson, was used as a standard in the present study. This substance is expensive, but is relatively stable and, furthermore, consistent in its results. To standardize the thiosulfate solution, 10 ml. of 0.01 normal bi-iodate was diluted to 50 ml. with sea water, alkaline-iodide and sulfuric acid added in that order, and the released iodine titrated as usual.

METABOLIC RATE UNDER VARIOUS CONDITIONS

Although oxygen uptake is not the only index of activity of an organism, it is correlated quantitatively with rate of metabolism and heat production and is the generally accepted standard measure of metabolic processes. In the present work the terms "metabolic rate", "oxygen consumption", and "oxygen uptake" will all be taken to mean the rate of oxygen consumption, expressed as cc./gram wet weight/hour. The following problems are taken up in this section: (1) the extent of normal fluctuations in rate of oxygen consumption and the presence or absence of

24-hour cycles, (2) the influence of temperature, and (3) the effect of different flow rates, in conjunction with varying quantities of fish, on the metabolic rate. The last problem is of particular interest to the local tuna fishery, since some of the boats are installing pumps capable of regulating the flow of water through the live-wells. To my knowledge, there have been no previous investigations concerned directly with the influence of flow rate on fish metabolism, excepting a short work by Washbourn (1936), who found that the rate of oxygen consumption of trout fry in swift-flowing water was significantly higher than in stagnant water. Wells (1935a) recognized the relation and cautioned that in any series of experiments on fish respiration the flow should be maintained invariable over long periods of time.

The relationship of rate of oxygen consumption to the oxygen concentration of the water itself will be discussed in a separate section.

Apparatus and General Methods

The flowing-water method was used in measuring rate of oxygen consumption, the principal advantage being the continual removal of carbon dioxide and other metabolic products given off by the fish. Essentially, this technique consists in delivering a stream of water to the experimental jar under a constant head of pressure, oxygen samples being taken at the inlet and outlet of the jar. Knowing the weight of the fish and the rate of flow in liters/hour, the rate of oxygen consumption may be readily calculated. Keys (1930b) compared the accuracy of the flowing-water method versus the sealed-jar method and found that, with the latter, few of his determinations could be duplicated to within 15 percent. With the apparatus used in the present study, consecutive determinations (less than 15 minutes apart) never varied more than 15 percent and generally showed closer agreement (see table 4a).

The respiration assembly is pictured diagrammatically in figure 2a. The large battery-jar reservoir (B) and overflow jar (C) allowed a constant head of water to be delivered to the experimental vessel at all times, and simultaneous oxygen samples could be taken by means of the inlet siphon from the reservoir (D) and the connection for outlet samples (F). With the exception of the flow-rate experiments, a 1-gallon wide-mouth jar was used as the experimental vessel. Figure 2b shows the water bath used in the temperature experiments. Immersed in the bath were a thermostatically controlled heating unit, motor driven stirrer, and a coil of copper tubing through which cold water was pumped to lower the temperature in the bath. The entire assembly rested in a Masonite box (not shown in the figure) packed with sawdust. Figure 2c shows the storage jar (about 10 inches in diameter) employed as an experimental container in the flow-rate experiments. A piece of Masonite served as the lid, being connected by threaded brass rods to another piece at the base of the jar. Leakage was prevented by forcing the lid down on a piece of rubber tubing.

In all the experiments oxygen samples were withdrawn in a standard fashion. Sampling bottles were placed simultaneously under (D) and (F) (fig. 2a) and sufficient time was allowed for one and one-half flushings of the bottles.

At the end of an experiment, the fish were weighed to the nearest 0.1 gram on a beam balance after first blotting them on paper towels to remove excess water. Since the validity of wet weight determinations depends on the constancy of the ratio of wet to dry weight, the dry weights of several samples of iao were determined. The samples were dried overnight at 70°-80° F., placed in a dessicator, and daily weighings were taken on a triple beam balance until a constant weight was reached in each of the samples. The percentage of dry weight to wet for each of the samples is shown in table 1 and is seen to vary only slightly. The lengths of all fish used in experiments were determined to the nearest millimeter, from the tip of the snout to the end of the vertebral column.

To determine the extent of oxygen consumption or oxygen production due to plankton in the sea water line, control experiments were run, with a 22-liter carboy as the "dummy" experimental jar. Oxygen samples from inlet and outlet were taken for several hours and a statistical analysis performed on the data (table 2). Although most of the trials showed a very small decrease in oxygen content, the difference was not statistically significant.

Table 1.--Ratios of dry weight to wet weight in iao

Sample No.	Number of fish	A (wet weight)	B (dry weight)	100 B / A
		gm.	gm.	percent
1	10	10.1	2.4	23
2	12	10.6	2.5	24
3	12	11.2	2.8	25
4	16	15.7	3.9	25
5	16	11.4	2.8	24
6	25	21.1	5.3	25
7	15	18.4	4.1	22
8	15	17.2	4.4	26
9	13	16.1	3.8	24
			Average:	24

Table 2.--Dissolved oxygen controls on the flowing-water apparatus used in measuring rate of oxygen consumption

Time	Determination No.	Temperature	Rate of flow	O_2 [1/]	
				Inlet	Outlet
		°C.	l./hr.	cc./l.	cc./l.
9:05 AM	1	23.8	14.52	4.64	4.66
9:45	2	24.0	14.52	4.62	4.62
10:30	3	23.8	14.52	4.64	4.66
11:00	4	23.8	14.52	4.69	4.66
11:35	5	23.8	14.52	4.78	4.72
1:00 PM	6	23.8	14.46	4.82	4.90
1:30	7	24.0	14.40	4.90	4.84
2:45	8	24.0	14.40	4.96	4.93
3:25	9	24.0	14.29	5.04	4.98
4:30	10	23.8	25.71	4.78	4.74
5:00	11	23.8	25.71	4.82	4.78
6:00	12	23.5	10.78	4.91	4.84
7:00	13	23.5	10.59	4.92	4.86

1/ All values are the average of two titrations.

Serial Determinations of Metabolic Rate: 24-Hour Experiments

Procedure.--Preliminary oxygen consumption tests indicated that a more or less consistent level of metabolic activity was reached after 1 to 2 hours in the container. It is not claimed that this represents a "basal" level of metabolism for iao, since the fish are not at complete rest, and furthermore it is obvious from the experiments described below that there is considerable fluctuation from hour to hour in the rate of oxygen uptake. It appears, however, that any great increase in metabolic rate due to the handling of the fish in setting up the experiment is dissipated after 1 to 2 hours.

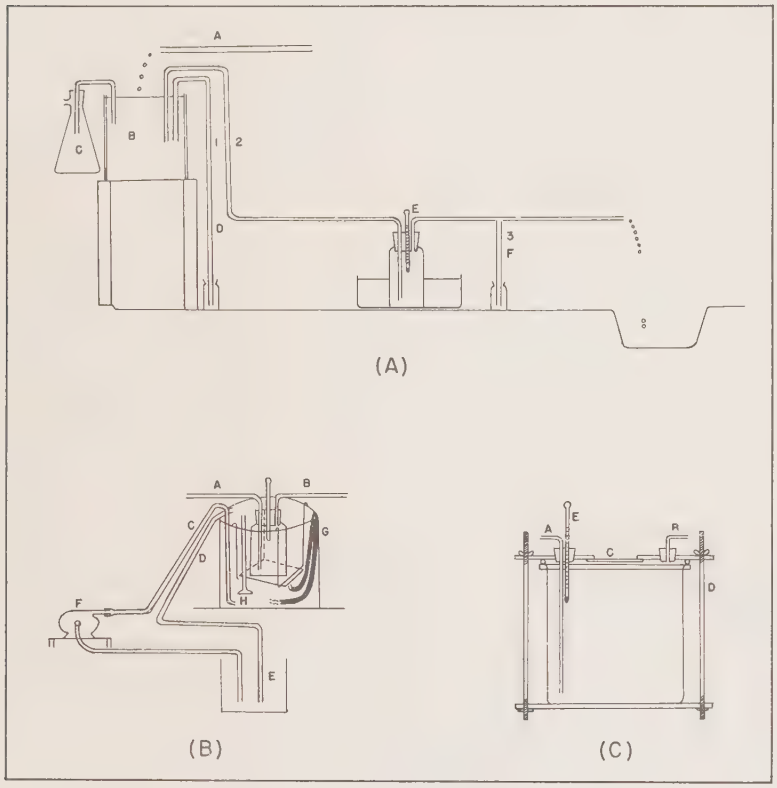

Figure 2a.-- Diagram of the apparatus used for measuring rate of oxygen consumption in iao. A - sea water line; B - reservoir; C - overflow jar; D - siphon for inlet samples; E - thermometer; F - connection for outlet samples. 1, 2, and 3: adjustable hose clamps.

Figure 2b.-- Assembly used for obtaining different temperature levels. A - inlet to experimental container; B - outlet from experimental container; C - inlet to copper coils; D - outlet from copper coils; E - cold bath; F - centrifugal pump; G - heating unit; H - stirring rod.

Figure 2c.-- Diagram of the experimental container used in the flow rate experiments. A - inlet to experimental container; B - outlet from experimental container; C - plastic observation window; D - threaded brass rod; E - thermometer.

In the actual experiments, 10 or more fish were carefully transferred to the jar and water was allowed to flow for 1 hour with the lid removed. The rubber cork with its connections was then inserted and the flow rate was adjusted to 12 liters/hour. Water samples were taken at 1-hour intervals for the first 3 or 4 hours, and approximately at 2-hour intervals thereafter.

Results.--The results of three successful 24-hour experiments, all performed in January and February of 1952, are reported in table 3 and figure 3. Although the data are quite variable, they seem to point to the absence of consistent diurnal cycles in the metabolic rate. In experiments 2 and 3, the rate of oxygen consumption fluctuates irregularly for about 6-8 hours, and then undergoes a slow, but nonetheless noticeable, decline for the remainder of the experiment. In both experiments, there is at the end of the 24 hours a small rise in the metabolic rate for which there is no apparent explanation. These tests, then, show only that there is an irregular, apparently non-cyclic, fluctuation in metabolic rate during the first 24 hours in the jar. Attention is called to the marked rise in oxygen consumption which occurred at

Table 3.--Serial measurements of metabolic rate (cc./gm./hr.) in iáo

Experiment 1

Number of fish: 18
Total weight: 15.9 gm.
Average length: 42 mm.
Range in length: 20-52 mm.

Time of day	Oxygen consumption	Temperature
	cc./gm./hr.	°C.
1215	.358	25.0
1315	.322	25.5
1410	.338	25.5
1800	.283	25.0
2300	.257	23.5
0215	.302	23.3
0640	.244	23.0
0835	.322	23.8
1045	.288	24.6
1150	.285	25.2

Experiment 2

Number of fish: 19
Total weight: 21.1 gm.
Average length: 47 mm.
Range in length: 40-55 mm.

Time of day	Oxygen consumption	Temperature
	cc./gm./hr.	°C.
1200	.288	25.0
1300	.230	25.4
1415	.298	25.0
1510	.313	25.0
1715	.269	25.0
2015	.426	24.5
2300	.263	24.0
0530	.258	23.5
0800	.204	24.0
1100	.209	24.4
1200	.230	24.5

Experiment 3

Number of fish: 20
Total weight: 21.1 gm.
Average length: 46 mm.
Range in length: 38-57 mm.

Time of day	Oxygen consumption	Temperature
	cc./gm./hr.	°C.
1100	.330	25.0
1200	.377	25.0
1300	.313	25.3
1515	.408	25.5
1700	.376	25.3
1915	.392	24.5
2100	.324	24.5
2400	.386	24.0
0300	.380	24.0
0600	.342	24.0
0915	.298	24.0
1115	.329	24.0

2000 hours in experiment 2. The fish might have been disturbed at this time because of frequent switching on and off of lights in adjacent rooms. The pattern for experiment 1 deviates from that of the other two experiments, possibly because of greater fluctuations in the temperature of the water (table 3). The results of this series, at any rate, are to be interpreted cautiously.

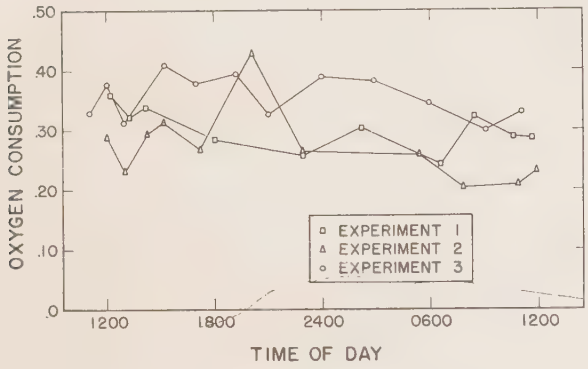

Figure 3.--Rate of oxygen consumption (cc./gm./hr.) in iao over a 24-hour period.

Influence of Temperature on Rate of Oxygen Consumption

Procedure.--With the arrangement shown in figure 2b, it was possible to raise the temperature in the container by approximately 2°C. intervals and keep it practically constant at any one level. The average variation in temperature was 0.3°C., the maximum variation in any test being 0.5°C. All experiments were begun at 19°C., at least 2 hours being taken to lower the temperature to this value. One set of samples was withdrawn at each temperature level, not less than 1/2 hour after the temperature reached the desired value. Fish used ranged in length from 34-50 mm., the average length of fish in each experiment ranging from 42-48 mm. The tests were run over a 10-day period in October 1951, during which time the temperature in the stock tanks was about 26°C.

Results.--The curve of temperature versus rate of oxygen consumption (fig. 4) resembles those found for other fish (e.g., Ege and Krogh 1914; Wells 1935a; Haugaard and Irving 1943). It rises slowly at first, the slope of the curve being somewhat steeper at the higher temperatures. Over the range in temperatures tested (19°-29°C.) there is an approximately twofold increase in the rate of oxygen consumption. Wells (1935a) has pointed out that small Fundulus show a more pronounced response to temperature changes than larger fish; however, the occasional presence of smaller iao (34-37 mm.) in the present experiments did not appear to alter the pattern of response to temperature. Knowing that the previous temperature history of the fish (acclimatization temperature) can markedly affect subsequent response to temperature changes (Wells 1935b, c; Sumner and Doudoroff 1938; Sumner and Lanham 1942), this factor was controlled as far as possible by running the experiments during one month (October) on fish which had been kept for some time at about 26°C.

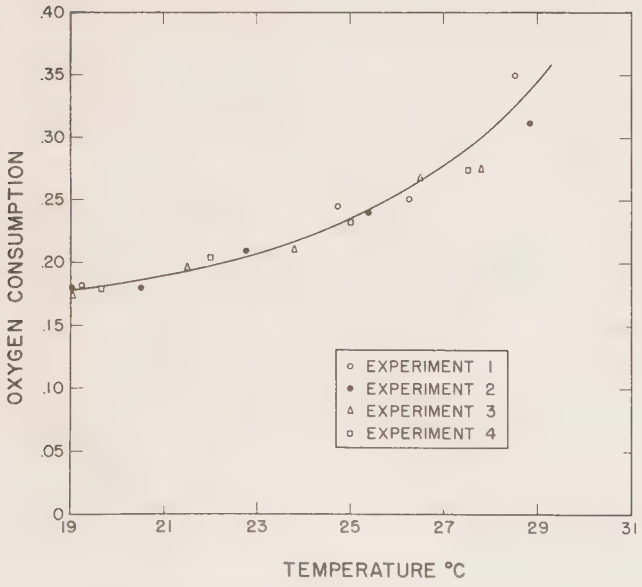

Figure 4.-- Rate of oxygen consumption (cc./gm./hr.) at different temperatures.

Influence of Flow Rate and Numbers of Fish upon the Metabolic Rate

Procedure.--In a typical experiment, the approximate desired number of fish were transferred to the experimental jar (fig. 2c), the flow was adjusted to an intermediate value, and about 1 hour was allowed for the fish to "settle down"

in the container. The lid was then inserted and the flow adjusted to the highest value tested. One hour later, two consecutive sets of oxygen samples (in a few instances only one set of samples was taken) were withdrawn, and the flow was then lowered to the next value in the series. Flows ranging from 7 to 37 liters/hour were tested in conjunction with aggregations of fish ranging in number from 20 to 132 or, in terms of concentration per unit volume, 7 to 44 fish per gallon. At the latter density the vessel was definitely crowded, but there was no indication of abnormal behavior, such as changes in schooling pattern, etc. by the fish. The experiments were run in the morning and early afternoon, with an average duration of 4 to 6 hours. All but three tests were performed in August and September (1951) at temperatures of 27°-28°C. The size of the container in itself appeared to keep the temperature from fluctuating and no temperature bath was deemed necessary. Fish of essentially the same size were used in all of the summer experiments, but somewhat larger fish were employed in the few winter tests.

Results.--The complete results are presented in table 4a. Figure 5 is drawn from the summer data and the points represent, where possible, the average of two values at each flow tested. The high values from experiment 4 are not included in the figure. In this experiment, the fish were initially extremely active and their behavior was not considered normal. Referring to figure 5 on the relation between flow rate and rate of oxygen consumption, curve 1 represents aggregations of 20 to 44 fish, curve 2 aggregations of 75 to 83 fish, and curve 3 applies to a single experiment using 132 fish.

For small aggregations of iao (20-45 fish), the rate of oxygen uptake is seen to increase linearly with increase in flow rate within the limits tested. At the lowest and highest flows, there is remarkable agreement in metabolic rate between the different lots of fish, with more variability evident at the intermediate flows. For comparable amounts of fish, tested in November (experiments 11 and 12), the influence of flow rate upon oxygen consumption is less striking; obviously, however, more experiments would be necessary to establish this point. The cause of this apparent difference in the behavior of summer and winter fish may be the cooler temperatures but attention is also called to the fact that larger fish were used.

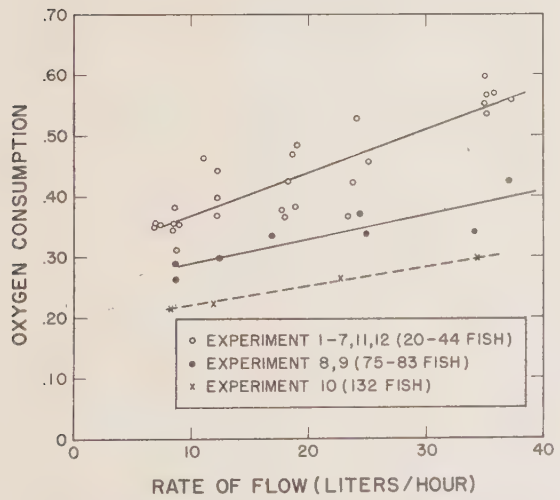

Figure 5.--Rate of oxygen consumption (cc./gm./hr.) in iao at different rates of flow (liters/hour). Regression coefficient for curve 1, .00735; for curve 2, .00412.

With 75 to 83 fish in the container, the relation of flow rate to oxygen consumption is much like that for small aggregations, though the effect appears to be less pronounced. More striking is the fact that the overall rate of oxygen uptake is lower. In experiment 10, where 132 fish were used, the level of oxygen consumption is again lower, but attention is called to the slightly lower temperature at which the experiment was performed.

A field trial, testing the influence of flow rates, was performed in the bait-well of the Territorial Fish and Game vessel Makua. Slightly over 2,000 grams of "mixed" bait (both nehu and iao) was used, giving a density of about 5 fish per gallon, roughly comparable to the minimum density used in the laboratory experiments (7 fish per gallon). The results, plotted in figure 6, show considerable fluctuation but bear some qualitative resemblance, at least, to the laboratory results. The large fluctuations observed may be due primarily to the inadequacies of field sampling.

Table 4a.--Rate of oxygen consumption (cc./gm./hr.) of iao at different rates of flow, in conjunction with varying quantities of fish. Experiments 1-10 were performed in August and September, and experiments 11 and 12 in November

Experiment No.	Number of fish	Total weight	Average length	Average temp.	Range in temp.	Rate of flow (l./hr.)					
						35-37	23-25	17-19	11-12	9	7-8
		gm.	mm.	°C.	°C.						
1	22	20.0	43	26.6	1.0	.588	-	.488	.476	.386	.354
						.606	-	.450	.447	.382	.350
						$\bar{x}$.597	-	.469	.462	.384	.352
2	39	26.5	41	26.9	1.0	.547	-	.424	.387	.348	.356
						.519	-	.424	.402	.351	-
						$\bar{x}$.533	-	.424	.395	.350	.356
3	30	21.7	42	28.0	0.5	.526	-	.343	.360	-	.345
						.571	-	.286	.372	-	.359
						$\bar{x}$.549	-	.315	.366	-	.352
4	26	19.0	42	27.6	0.7	?.893	.487	.481	-	.355	-
						?.964	.554	.481	-	.355	-
						$\bar{x}$.929	.521	.481	-	.355	-
5	44	28.0	41	28.3	0.5	.554	.465	.360	.428	Temp. rising, fish excited	
						.579	.438	.367	.460		
						$\bar{x}$.567	.452	.364	.444		
6	20	15.2	42	27.3	0.8	.533	.383	.400	-	.358	-
						.580	.353	.362	-	.335	-
						$\bar{x}$.557	.368	.381	-	.347	-
7	28	21.0	42	27.0	0.4	.543	.429	.374	-	.313	-
						.578	.417	.381	-	.309	-
						$\bar{x}$.561	.423	.378	-	.311	-
8	75	55.2	41	28.4	0.0	.426	.367	-	.299	.291	-
						.419	.372	-	.298	.290	-
						$\bar{x}$.422	.370	-	.298	.290	-
9	83	51.4	39	28.4	0.1	.333	.338	.331	-	.263	-
						.346	.338	.334	-	-	-
						$\bar{x}$.340	.338	.332	-	.263	-
10	132	116.3	43	26.1	0.2	.289	.263	-	.220	.215	-
						.295	-	-	-	-	-
						$\bar{x}$.292	.263	-	.220	.215	-
11	30	34.5	47	24.8	0.1	.285	.300	-	.246	.241	-
						.285	-	-	-	-	-
						$\bar{x}$.285	.300	-	.246	.241	-
12	35	44.4	48	24.3	0.7	.242	.218	-	.197	.183	-
						.220	-	-	-	-	-
						$\bar{x}$.231	.218	-	.197	.183	-

Discussion

A striking feature of the serial observations of rate of oxygen consumption (table 3) is the great variability from hour to hour. Such fluctuations are apparently not uncommon, however, having been observed by several workers (Chapman 1939; Clausen 1936; Shlaifer 1939; Wells 1935a). The following statement of Wells (1935a) is perhaps apropos: "Duplication of results with the same animals at two different times is not a measure of the efficiency of the apparatus, nor is a failure to duplicate results a reflection upon the method." Krogh (1916) mentions that the respiratory exchange in a normal animal may vary 100 percent or more. Over the 24-hour period during which serial observations were made, no consistent rhythms in metabolic rate were evident. Daily cycles in metabolic rate have been claimed for a few freshwater fish (Clausen 1936; Oya and Kimata 1938; Higginbotham 1947) but have not received extensive confirmation.

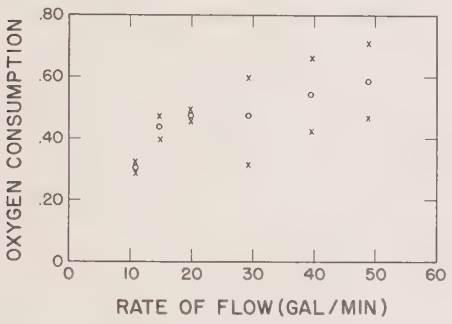

Figure 6.--Results of a field experiment testing the influence of flow rate (gal./min.) on rate of oxygen consumption (cc./gm./hr.). Open circles represent the average of two determinations of oxygen consumption (represented by crosses) at each flow.

The temperature-oxygen consumption curve (fig. 4) in general resembles those for other fish but must, of course, be interpreted entirely upon its own merits, particularly since a relatively narrow temperature range was studied. The curve may be said to represent an experimentally determined relationship of temperature to rate of oxygen consumption in iao ranging in size from 34 to 50 mm. for the temperature interval 19°-29°C. In view of the apparently valid arguments of Ege and Krogh (1914) and Wells (1935a) against the unrestricted use of Van't Hoff's Q_{10} and the Arrehenius relation, no attempt was made to calculate either Q_{10} values or "thermal increments." Ege and Krogh, for example, found that Q_{10} varied from 9.8 at 0°-5°C. to 2.2 at 23°-28°C. in the goldfish.

At the higher temperatures in the range tested, the effect of temperature upon metabolic rate appears somewhat more pronounced. Since the local fishery is conducted almost entirely during the summer and early fall, when water temperatures often rise to 27°-28°C. and higher, it may be advisable to watch for sudden changes in the water temperature of the live-wells. Even a small rise in temperature might considerably increase the activity and consequently the rate of oxygen consumption of the fish.

That the rate of oxygen consumption should rise with increase in rate of flow is perhaps not surprising, inasmuch as the fish would presumably exert more energy in swimming as the current of water through the jar increases in intensity. In curve 1 (fig. 5), representing small aggregations of fish and based on the most extensive collection of data, oxygen consumption increases linearly with flow rate over the range from 7 to 37 liters/hour. The points appear less scattered at the extreme flow values. This may be a chance variation or the fish, for some reason, may exhibit a uniform metabolic response to the environment at the extreme flow rates. This explanation is easier to accept in the case of the high flow values (35-37 liters/hour), where the fish might all be exerting approximately the same amount of energy in maintaining their position against the strong current.

When larger numbers of fish are used (curve 2, fig. 5), the overall level of metabolism is lower. An analysis of covariance (tables 4b and 4c) on the data making up curves 1 and 2 yields the following statistical conclusions: 1) the difference between the mean values for metabolic rate, adjusted to a common flow value, of group 1 (20-45 fish, adjusted mean 0.431 cc./gm./hr.) and group 2 (75-83 fish, adjusted mean 0.320 cc./gm./hr.) is highly significant ($P < 0.01$), indicating

Table 4b.--Analysis of covariance and test of significance of adjusted mean oxygen consumption values for group 1 (20-45 fish) and group 2 (75-83 fish)

Source of variation	Degrees of freedom	Errors of estimate		
		Sum of squares	Degrees of freedom	Mean square
Total	36	0.145	35	-
Groups	1	-	-	-
Within groups (error)	35	0.069	34	0.0020
For testing significance of adjusted means	-	0.076	1	0.076 **

**Significant at 1-percent level ($P < 0.01$).

Table 4c.--Analysis of errors of estimate from the average regression within groups

Source of variation	Degrees of freedom	Errors of estimate	
		Sum of squares	Mean square
Deviations from average (error) regression within groups, table 4b	34	0.069	-
Deviations from individual groups regressions	33	0.063	0.0019
Differences among group regressions	1	0.006	0.006

that the drop in oxygen consumption is probably a real one; and 2) the difference between the slopes of the two curves is not significant (table 4c), indicating that the effect of flow rate on oxygen consumption is similar regardless of the numbers of fish tested.

The drop in metabolic rate with larger groups of fish deserves further discussion. Schuett (1933) and Shlaifer (1938) both point out that 2 to 4 goldfish in a container consume significantly less oxygen per unit weight than single fish. A similar "group effect" has been observed by Geyer and Mann (1939a, b) in Perca fluviatilis. All these authors find that the effect disappears when the size of the container is increased in proportion to the increase in numbers of fish. The present data are inadequate to justify anything but tentative conclusions. The decrease in metabolic activity is perhaps due to changing intensity of motion caused by the reduction in space available per fish. No evidence is available, however, attesting to the possible influence on the

metabolic rate of accumulated excretory products and other gases, particularly when large numbers of fish are used. This aspect should be investigated further. Geyer and Mann (1939a) are of the opinion that a decrease in oxygen concentration with greater numbers of fish is responsible for the "group effect." This factor can probably be neglected in the present work since, as will be pointed out in the next section, oxygen consumption in the iao is independent of concentration down to at least 2.5 cc./l. In the flow-rate experiments, the oxygen concentration of the outlet samples in no case fell below 2.5 cc./l.

It is of interest to evaluate these laboratory findings in terms of the problems involved in keeping bait alive in fishing vessels. The lowering of metabolic rate with large numbers of fish might be construed to mean that increasing the number of fish in a live-well leads to more efficient maintenance. This may be true, but more data are necessary to substantiate such a view. Furthermore, if too many fish are present, the oxygen content of the water obviously may be reduced to near the lethal value, which, as will be seen shortly, is about 1.0 cc./l. for iao. For nehu, the preferred baitfish, the limited data available indicate a lethal value of about 2.0 cc./l. (table 7). Those boats having pumps could handle more fish by increasing the flow rate through the live-wells, but this in itself causes an increase in activity and oxygen consumption (fig. 5). In order to arrive at the best combination of flow rates and aggregation size the present laboratory experiments should be followed up by field tests, possibly using the same flow rates and densities of fish.

METABOLIC REGULATION IN THE IAO

In summarizing the literature pertaining to the relationship between rate of oxygen consumption and the dissolved oxygen content of the water, it is apparent that some fish are markedly affected by changes in external oxygen while others are not. Gaarder (1918) found a very slight dependence of oxygen consumption on oxygen concentration in the carp. Powers and Shipe (1928) claim that for herring, coho salmon, and viviparous perch a decrease in the amount of dissolved oxygen caused a proportional decrease in the rate of oxygen consumption. According to Hall (1929), the degree of metabolic regulation depends on whether the fish is an active or sluggish species, the former showing metabolic independence and the latter complete dependence on the oxygen concentration of the medium. In any event, small changes from the normal oxygen content of the water probably do not affect oxygen uptake in most fish (Keys 1930a; Wells 1935a; Lindroth 1940; Fry and Hart 1948), but an oxygen level is eventually attained below which metabolic adjustment fails and the rate of oxygen uptake begins to decrease. This "critical level" varies with species and with temperature (Lindroth 1940; Fry and Hart 1948; Graham 1949).

One means by which fish accomplish metabolic regulation is an increase in the frequency of breathing movements. This compensates for the fall in oxygen concentration by permitting more water per unit time to flow past the gill surfaces. Several workers (Westerlund 1906; Winterstein 1908; Wells 1913; Gardner and King 1922; Belding 1929; Wilding 1939) have incidentally observed an increase in the rate of opercular movement, to a greater or lesser degree, with a decrease in the oxygen content of the water. On the other hand, there are few works in which both the rate of oxygen consumption and breathing movements are analyzed and their interrelations discussed. Notable exceptions are papers by Meyer (1935), who worked with Uranoscopus scaber, and van Dam (1938), who worked on eel and trout. Both workers determined not only breathing rate, but breathing depth and ventilation volume in relation to oxygen concentration. Van Dam found that the eel (Anguilla vulgaris) showed much better metabolic regulation than the trout (Salmo shasta).

The above discussion points to the importance of determining the degree of metabolic regulation in any fish whose oxygen relations are being investigated. In this section the relationship of metabolic rate to oxygen concentration is analyzed, and to supplement these data the rate of opercular beat is determined at various oxygen values.

Apparatus

To introduce water of different oxygen concentrations, several modifications in the basic experimental assembly were necessary. Figure 7 shows the modified assembly set up for experiments on breathing movements. When the apparatus was used to determine rate of oxygen consumption, a wide-mouth gallon jar was substituted for the glass tube shown suspended in the water bath. The carboy on the left contained water low in oxygen, obtained by bubbling through nitrogen gas, and the one on the right contained normal sea water. Water from both carboys was joined by a "Y" tube into a single flow entering the overflow flask. Oxygen samples were taken in the manner described earlier, with the exception that inlet samples were siphoned from the overflow flask. Control tests indicated only negligible variation in dissolved oxygen content between carboy, overflow flask, and the outlet of the experimental chamber.

Relation between Dissolved Oxygen Concentration and the Rate of Oxygen Consumption of the Iao

Procedure.--Since the supply of low-oxygen water from the carboy was limited, the plan was to test several lots of fish, each at a different oxygen level, from 7 to 14 fish being used in each experiment. After the fish were in the container, normal sea water was circulated through the jar for 1 hour. Two consecutive sets of oxygen samples were then taken from which the average rate of oxygen consumption in normal sea water, i.e., the "control" value, was calculated. The normal sea water line was then pinched off and oxygen-poor water was permitted to circulate through the system. After 30 minutes, a set of oxygen samples was taken and the metabolic rate at the new oxygen level was determined.

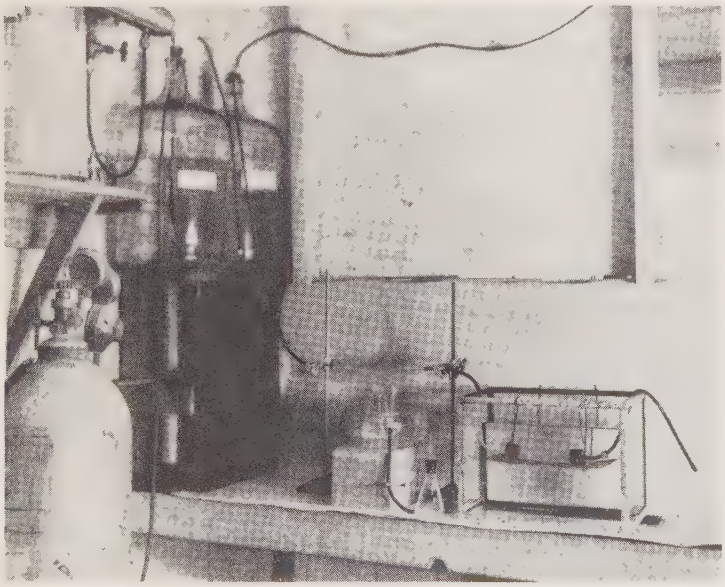

Figure 7.--Apparatus used in measuring oxygen consumption and breathing rate at different oxygen concentrations. The apparatus is shown assembled for breathing rate experiments. Detailed description in text.

The experiments were all performed during morning hours at temperatures generally between 24° and 25°C. A fairly wide range of sizes was employed, the average length of fish in each test ranging from 37 to 49 mm.

Results.--The relationship between oxygen consumption and oxygen concentration (fig. 8) shows that the rate of oxygen uptake fluctuates about a constant level down to low values of oxygen but eventually starts to fall, the decline becoming more rapid as the lethal value of oxygen is approached. Complete data are reported in table 5. Unfortunately, few points are available in the region where the decline in oxygen consumption begins and it is difficult to establish anything like a definite "critical" level of oxygen. At best, it can be stated that the critical level lies between 1.5 and 2.5 cc./l. Some of the scattering of points, particularly in the first part of the curve, may be attributed in part to the rather large range of sizes used. On the other hand, tests in which a great many small fish were used did not indicate, on the whole, a greater or lesser decrease in oxygen consumption compared with tests in which many large fish were used. (Compare, for example, experiments 7 and 14, table 5.) Since the experiments were performed over a fairly narrow range of temperatures, it appears unlikely that the critical level would be shifted due to differences in temperature between tests.

Table 5.--Rate of oxygen consumption (cc./gm./hr.) at various oxygen concentrations

Experiment No.	Total weight of fish used	Range in length	Average length	Temperature	Average control O_2 value	Rate of oxygen consumption (control)	Average experimental O_2 value	Rate of oxygen consumption (experimental)	Decrease in consumption from control
	gm.	cm.	cm.	°C.	cc./l.	cc./gm./hr.	cc./l.	cc./gm./hr.	cc./gm./hr.
1	8.1	44-57	49	23.5	4.21	.330	1.15	.079	.251
2	7.0	41-54	44	23.6	4.13	.428	1.09	.166	.262
3	6.8	44-56	48	24.0	4.14	.354	1.14	.107	.247
4	9.1	41-51	46	24.8	4.06	.417	1.25	.204	.213
5	10.6	42-59	47	24.4	3.93	.374	3.42	.304	.070
6	10.0	36-51	45	25.0	3.95	.522	1.12	.209	.313
7	12.4	36-52	44	25.0	3.82	.506	2.66	.369	.137
8	9.6	36-55	44	25.0	3.96	.416	2.22	.292	.124
9	9.0	35-52	46	25.2	4.12	.446	3.02	.394	.054
10	9.9	38-56	46	24.2	4.08	.448	3.05	.358	.090
11	8.2	30-51	46	24.8	3.91	.423	3.86	.346	.077
12	8.0	36-50	45	25.6	4.09	.437	3.90	.393	.044
13	9.7	43-51	48	24.0	4.07	.353	1.14	.144	.209
14	6.1	32-49	39	25.5	3.86	.484	1.12	.161	.323
15	5.3	33-44	39	26.0	3.82	.478	1.44	.308	.170
16	5.6	34-45	37	25.8	4.09	.420	2.71	.328	.092
17	7.2	38-52	39	26.8	5.39	.417	3.10	.417	.000
18	6.9	35-46	41	25.0	3.86	.448	1.58	.258	.190
19	9.3	33-52	41	24.5	3.88	.347	1.84	.261	.086

Frequency of Opercular Movements in Iao at Different Oxygen Concentrations

Procedure.--To obtain accurate gill movement counts, it was necessary to immobilize the fish, which was done by placing it in a clay mount on a microscope slide with only the head and gill cover protruding. With practice in the technique, handling could be reduced to a minimum. The immobilized fish was placed in a finger bowl of sea water and, if it did not work free and if it assumed a regular opercular beat, it was quickly transferred to the glass tube immersed in the water bath. Normal sea water was allowed to flow through the tube for about 30 minutes before counts were taken. In these experiments it was possible to test an individual fish at more than one oxygen level, the different levels being obtained by mixing appropriate amounts of water from the two carboys. Five determinations of the time required for 50 beats (from which the beats/minute was calculated) were taken at each oxygen value.

The reliability of the method was tested by taking counts in normal sea water over a 90-minute period (the maximum duration of any experiment) on two fish. The results indicated little fluctuation in normal opercular rhythm. For each fish 15 separate counts were taken, the means and standard deviations being 215 ± 4.2 (fish 1) and 213 ± 3.7 (fish 2).

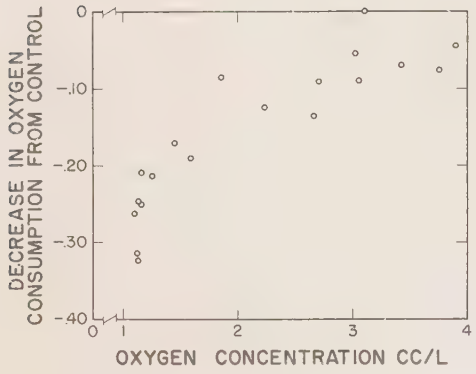

Figure 8.--Change in rate of oxygen consumption (cc./gm./hr.) with a decrease in the oxygen content of the water. Ordinate represents the decrease in rate of oxygen consumption from control values.

The experiments were performed in February of 1952 and 1953, at temperatures of 23°-24°C. (average 23.9°C.). Fish used ranged in length from 39 to 62 mm. and in weight from 0.56 to 2.46 gm., weight being taken to the nearest 0.01 gm. on a Roller-Smith torsion balance after 1 week's preservation in 10-percent formalin.

Results.--From the eye-fitted curve (fig. 9) it is seen that as the oxygen content of the water falls, the respiratory rate rises, slowly at first, then more rapidly, to a peak beyond which it slowly decreases. The maximum breathing rate is attained probably somewhere between 2.0 and 2.5 cc./l.--the peak in the curve is not well defined, principally because of the very gradual subsequent decrease in breathing rate. The points are widely scattered in the first part of the curve and this is reflected in the behavior of the fish. As the oxygen concentration is lowered the fish at first show some agitation and occasional irregular breathing. Later, at the very low oxygen values, breathing is slower and more regular. No quantitative measurements of breathing-depth were made, but the fish were observed to breathe deeper, as well as faster, as the oxygen content was lowered. Deep breathing continued, in several cases, after breathing frequency began to diminish.

Discussion

Comparison of the relationship of oxygen consumption (fig. 8) and opercular rhythm (fig. 9) to the oxygen content of the water is experimentally difficult for at least two reasons. For one thing, it was necessary to use different techniques in each series of tests. In the experiments on oxygen consumption each group of fish could be tested at only one oxygen level, whereas in the breathing rate experiments a fish could be run through a whole series of oxygen values. Furthermore, the points are somewhat scattered in both cases, especially at the higher oxygen concentrations, and it is difficult to determine precise oxygen values below which rate of oxygen consumption and breathing rate decline. With these precautions in mind, it is still perhaps safe to say that both breathing rate and oxygen consumption begin to decrease at approximately the same level of oxygen, somewhere between 1.5 and 2.5 cc./l., the implication being that the fall in breathing rate contributes, in part at least, to the fall in oxygen consumption. From the standpoint of the livebait fishery, a "danger" point might be placed at 2.5 cc./l. If the oxygen content of the water in the live-wells falls much below this value, the fish will more than likely not be getting sufficient oxygen to maintain normal metabolism.

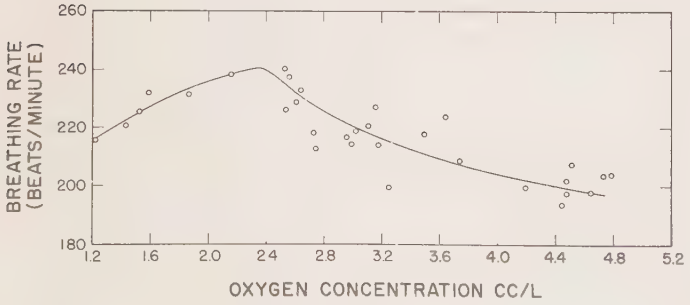

Figure 9.--Breathing rates (beats/minute) at different oxygen concentrations.

LETHAL VALUES OF OXYGEN

A major problem in the confinement of baitfish is the possible depletion of oxygen to a level below that tolerated by the fish. Suehiro (1951) found that newly introduced "wild" bait congregated densely at the surface, and a localized depletion of oxygen to below the lethal value occurred, regardless of an increase in the

amount of water circulated through the tank. Information on lethal values of oxygen is widely scattered through the literature and there are few extensive experimental investigations of the subject. In view of these considerations, it was thought worthwhile to determine the lethal value of oxygen for the local species of baitfish. Both individual fish and groups of 10 to 15 fish were asphyxiated in closed containers. In addition, the effect of temperature on the lethal value of oxygen was determined, single fish being employed for these tests. Complete cessation of all movements, breathing or otherwise, was taken as indicating the death of the fish, the validity of this criterion being evidenced by the fact that no recoveries were obtained upon return to normal sea water.

With the exception of the first summer's experiments, all oxygen samples were analyzed by the Rideal-Stewart modification of Winkler's procedure. A comparison between the modified and unmodified methods is reported in table 6. The oxygen samples were siphoned off simultaneously from large, sealed Erlenmeyer flasks in which fish had been using up the dissolved oxygen for some time. The mean difference in the values obtained by the two methods is not significant (P = 0.08), but the discrepancy in some of the trials is pronounced; to be safe, therefore, it was decided to use the Rideal-Stewart procedure.

Table 6.--A comparison of the Rideal-Stewart and unmodified Winkler procedures for determining the dissolved oxygen content of water in which fish have extensively respired

Experiment No.	Number of fish	Oxygen at end of experiment (cc./l.)		
		Rideal-Stewart	Unmodified	Difference
1	5	2.40	2.50	-0.10
2	6	2.48	2.46	0.02
3	7	1.45	1.38	0.07
4	4	3.08	3.06	0.02
5	4	1.38	1.08	0.30
6	4	2.46	2.48	-0.02
7	3	1.02	0.94	0.08
8	3	1.30	1.16	0.14
9	2	1.28	1.20	0.08
10	3	1.32	1.24	0.08
11	4	1.10	1.12	-0.02

Mean = 0.059

Standard Error of the Mean = 0.031

"t" = 1.90

P = 0.08

Lethal Values of Oxygen for Nehu and Iao: Experiments Using Single Fish

Procedure.--For most of these tests Florence flasks of about 540 ml. capacity were employed as experimental containers. Occasionally, depending on the size of fish used, larger or smaller flasks were necessary. The flasks were surrounded by white cloth to prevent the fish from becoming unduly excited, and a small piece of screening was placed in the neck to keep the fish from entering this portion of the flask.

After the fish was transferred to it, the flask was filled, stoppered, and immersed to its neck in a water bath. Initial oxygen samples were obtained from flasks immersed in the water bath but containing no fish. After all movement of the fish in the experimental jar had ceased, an oxygen sample was quickly siphoned off, the water being allowed to circulate through the bottle for a few seconds after it was filled. In some of the earlier experiments a 50-ml. sample was then taken for pH determination, leaving about 50-70 ml. in the flask. The length of the fish, from the tip of the snout to the end of the vertebral column, was taken to the nearest millimeter, and with the exception of some of the earlier experiments, the fish were preserved in 10-percent formalin to be weighed later on a Roller-Smith torsion balance.

As a check on the possibility of errors due to absorption of oxygen during siphoning, water samples of low oxygen content were siphoned off from two flasks, one with a layer of mineral oil and the other without the layer. The difference in amount of dissolved oxygen between these samples was within the error of the Winkler procedure.

The experiments on iao were divided into two groups, a summer series and a winter series, reported respectively in tables 8a and 8b. Eleven experiments were performed with nehu, all in the late spring and summer of 1950, and these are reported in table 7.

Results.--Considerable variability was evident in the results, as might be expected in collecting data on individuals. The mean and standard deviation of all the summer lethal values for iao (table 8a) was 1.10 ± 0.22 cc./l. and that of the winter values 0.96 ± 0.18 cc./l., with mean temperatures of $25.6 \pm 0.9^{\circ}$C. and $21.9 \pm 0.6^{\circ}$C. respectively. The difference between these mean values, when tested statistically by analysis of variance (table 8c), was found to be significant at the $P = 0.05$ level. The mean lethal value for nehu was 2.02 cc./l. with a standard deviation of 0.35 cc./l., much larger than that for either series on iao. The great variability of the nehu data was probably a consequence of the fish's behavior when confined to the experimental flask. In contrast to iao, nehu generally remained greatly agitated throughout the experiment and some undoubtedly became injured, thus causing an abnormally high lethal value of oxygen.

Weights were not available for all the fish used, but the data on hand show no significant relationship between weight of fish and the lethal value of oxygen. Regression lines for both summer and winter data did not differ significantly from zero ($P = 0.1$ in both cases). At best, a slight trend towards greater resistance to oxygen deficiency in the larger fish was indicated.

It seems fairly certain that the death of the fish in these tests was due to lack of sufficient oxygen and was not affected by a lowering of the pH during the course of the experiment. Hydrogen ion concentration was measured at the end of the earlier experiments and was never less than 7.80, averaging 7.87. The water in the stock tanks often showed pH values of this magnitude.

Some evidence is available (experiments 16, 17, and 18 of the winter series) to indicate that lethal values of oxygen are about the same regardless of the initial oxygen content of the water. This point, however, needs further investigating.

Table 7.--Lethal values of oxygen for nehu

Date	Experiment No.	Temperature	Duration of experiment	O_2 (cc./l.)		Volume of jar	Length of fish
				Initial	Final		
		°C.				cc.	mm.
3-9-50	*1	22.9	1hr40min	5.04	2.54	1070	-
3-17-50	*2	24.7	1hr45min	4.95	2.42	"	-
3-18-50	*3	25.0	2hr15min	5.36	1.87	"	-
3-21-50	*4	24.8	1hr47min	4.90	2.41	535	-
4-22-50	*5	26.5	2hr	3.91	2.30	"	-
8-23-50	6	25.6	4hr35min	4.73	1.96	"	34
8-24-50	7	25.0	3hr55min	4.68	1.64	"	37
"	8	"	7hr10min	"	1.60	"	31
"	9	"	4hr45min	"	1.66	"	35
8-25-50	10	24.8	6hr40min	4.36	1.80	"	29
"	11	"	3hr45min	"	1.98	"	34
				mean 2.02 $\pm$ .35			

*Experiments performed at the Waikiki Marine Laboratory.

Table 8a.--Summer lethal values of oxygen for iao

Experiment No.	Temperature	Duration of experiment	O_2 (cc./l.)		Volume of jar	Weight of fish	Length of fish
			Initial	Final			
	°C.				cc.	gm.	mm.
*1	25.9	1hr12min	3.69	1.05	1070	-	-
*2	26.2	1hr22min	5.32	1.27	"	-	-
*3	25.7	1hr30min	6.20	1.38	"	-	-
*4	24.4	1hr05min	5.17	1.01	"	-	-
*5	24.5	1hr23min	5.48	1.28	"	-	-
6	27.3	3hr55min	4.60	1.58	535	-	37
7	27.0	6hr15min	4.50	1.28	"	-	30
8	24.3	8hr55min	4.67	0.94	"	-	25
9	24.0	7hr50min	4.67	1.11	"	-	28
10	26.0	6hr30min	4.72	1.20	"	-	31
11	27.1	2hr	4.38	0.87	"	-	58
12	27.1	2hr45min	4.38	0.86	"	-	50
13	25.3	2hr50min	4.44	1.08	"	1.06	45
14	25.3	1hr30min	4.44	0.75	"	2.80	60
15	25.3	3hr30min	4.44	1.17	"	0.75	41
16	26.5	3hr	4.43	1.41	250	0.57	39
17	25.0	3hr55min	4.36	1.02	"	0.43	36
18	25.2	6hr45min	4.33	1.18	535	0.60	37
19	25.2	4hr	4.33	1.20	"	0.96	44
20	25.1	5hr30min	4.50	0.63	"	1.10	45
21	25.1	4hr15min	4.50	1.18	"	1.10	46
22	25.1	6hr25min	4.50	0.88	"	0.80	42
23	25.1	3hr25min	4.50	1.07	"	1.34	49
mean	25.6 $\pm$ 0.9		1.10 $\pm$ 0.22				

*Experiments performed at the Waikiki Marine Laboratory.

Table 8b.--Winter lethal values of oxygen for iao

Experiment No.	Temperature	Duration of experiment	O_2 (cc./l.)		Volume of jar	Weight of fish	Length of fish
			Initial	Final			
	°C.				cc.	gm.	mm.
1	23.1	2hr45min	4.60	0.96	535	2.09	54
2	23.1	6hr	4.60	0.82	"	1.08	45
3	23.1	5hr15min	4.60	0.80	"	1.36	48
4	21.3	3hr30min	4.94	1.03	"	1.66	51
5	21.3	4hr30min	4.94	0.86	"	1.52	50
6	21.9	5hr	4.70	1.16	"	1.14	46
7	21.9	5hr05min	4.70	1.14	"	1.49	50
8	21.9	4hr10min	4.70	1.04	"	1.45	50
9	21.6	3hr10min	4.58	1.17	"	1.50	49
10	21.6	3hr10min	4.58	1.16	"	1.49	49
11	21.6	3hr45min	4.58	1.09	"	1.37	48
12	21.6	4hr30min	4.58	0.90	"	1.25	47
13	21.6	4hr05min	4.58	1.21	"	1.00	45
14	21.6	4hr20min	4.58	0.79	"	1.36	49
15	21.4	4hr15min	4.15	0.82	"	1.95	52
16	21.4	45min	1.10	1.02	"	1.06	43
17	21.4	3hr25min	2.01	0.78	"	1.35	47
18	22.3	4hr15min	2.96	0.50	"	1.98	53
mean	$21.9 \pm .6$			$0.96 \pm .18$			

Table 8c.--Analysis of variance of the data from the above two tables

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Total	40	1.8995	-
Seasons	1	0.2152	0.2152*
Within seasons	39	1.6843	0.0431

*Significant at 5-percent level ($P < 0.05$).

Lethal Values of Oxygen for Iao: Experiments Using Several Fish

Procedure.--In these experiments, 10-15 fish (usually 14) were placed in a 1-liter aspirator jar with a side-opening close to the bottom for withdrawing water samples. Water was circulated through the container for 1 hour, at which time the fish were quiescent and appeared to be schooling normally. Circulation was then stopped, about 100 ml. was run out and replaced by mineral oil, after which the jar was securely stoppered. Three samples were taken in each experiment, after approximately one-third, one-half, and all of the fish had died. At the end of the experiment all fish were measured to the nearest millimeter. The tests were run in December 1951 and January 1952.

Results.--Table 9 gives the conditions and results of each experiment, and figure 10 shows graphically the relation between oxygen concentration and percentage of fish dead, the curve being fitted by the method of least squares, using a log-log transformation of the data. For those experiments employing 14 fish, the times to approximately 50-percent mortality are fairly similar, indicating more or less uniform behavior for the different lots of fish. Comparing the data with the winter lethal values of oxygen obtained from individual fish (table 8b), it is evident that the overall range in lethal values is nearly the same, being 0.55-1.30 and 0.50-1.21 cc./l. respectively. On the other hand, the mean value from table 8b (0.96 cc./l.) is higher than the mean of the values from the present experiments. Inspection of figure 10 indicates that, in the group experiments, practically all the fish died at oxygen concentrations between 0.55 and 0.85 cc./l. whereas figure 11, plotted from the data of table 8b, indicates, for the tests on individuals, a more even distribution over the range of lethal values of oxygen.

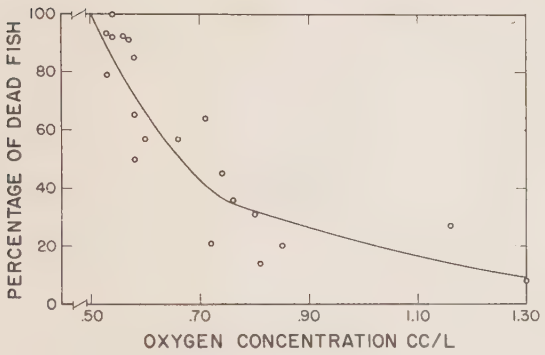

Figure 10.--Lethal values of oxygen for iao: experiments using several fish. Abcissa represents the oxygen content of the water; ordinate represents the percentage of fish dead at the various oxygen concentrations.

Effect of Temperature on Lethal Values of Oxygen for Iao

Procedure.--These tests, in which single fish were used, were performed in a constant temperature apparatus consisting of six compartments separated by tin plates with a thermostatically controlled heating coil in one of the end compartments and a similarly controlled refrigeration unit in the other. The water in the baths was agitated at intervals by compressed air. After allowing 4-5 hours to establish a constant gradient of temperature, the temperature in each of the baths rarely fluctuated more than 0.3°-0.4°C., with the hotter baths in general showing the most variability.

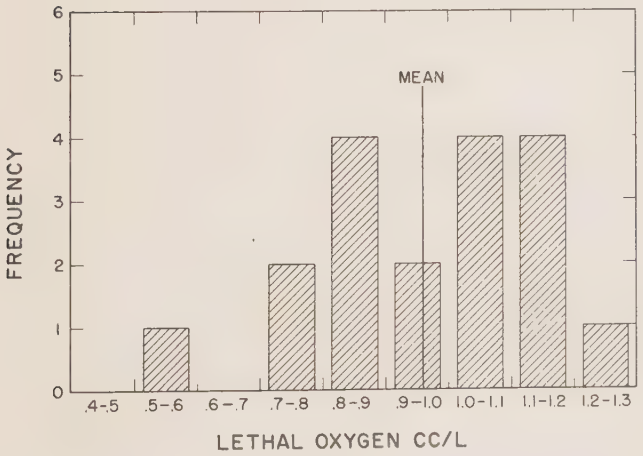

Figure 11.--Frequency distribution of winter lethal values of oxygen for iao, calculated from data of table 8b.

In the experiments, two Florence flasks, each containing one fish, were immersed in each temperature bath. Handling of fish and water sampling were done in the manner described in the previous sections. Excepting a few experiments at 18°C., all tests were made at temperatures ranging from 21° to 31.5°C. Preliminary tests indicated that iao die almost immediately at temperatures over 34°C. but, with the exception of a great increase in activity, appear to behave normally at 31°C. For the tests at 18°C. the fish were first gradually introduced to this temperature by placing a gallon jar containing five fish in the water bath used for the temperature versus oxygen consumption experiments (fig. 2b) and circulating cold water through the copper coils while a slow stream of sea water flowed through the jar. Final equilibrium at 18°-19°C. was reached after 3 to 4 hours, thus avoiding the shock of a sudden

transfer to colder water. After 24 hours at 18°-19°C. no fish had died and all appeared to be in good condition.

Table 9.--Lethal values of oxygen for iao: experiments using several fish

Date	Experiment No.	Number of fish	Average length	Percent dead	O_2	Time for approx. 50% of fish to die	Temperature
			mm.		cc./l.		°C.
12-8-51	1	11	45	27 64 91	1.16 0.71 0.57	-	23.8
12-11-51	2	14	43	21 57 100	0.72 0.66 0.54	1hr50min	23.0
12-16-51	3	13	48	8 31 85	1.30 0.80 0.58	-	23.0
12-18-51	4	14	45	36 79 100	0.76 0.53 0.54	1hr40min	23.0
12-28-51	5	14	43	14 50 92	0.81 0.58 0.54	2hr15min	23.4
12-29-51	6	14	46	92*	0.56	2hr10min	22.4
1-3-52	7	10	49	45 65 100	0.74 0.58 0.54	-	23.4
1-15-52	8	15	45	20 57 93	0.85 0.60 0.53	-	22.0

*All fish died within 5-10 minutes of each other.

Results.--The experiments were performed in January and February of 1951 and of 1952 and the fish used were therefore taken from waters ranging in temperature from 22° to 23°C. The results are presented in figure 12 and table 10, the points on the graph representing the average value for the two flasks in each bath. Lethal values of oxygen are seen to increase slowly at first (between 20.5° and 23°C.) and then more rapidly above 26°C. In spite of considerable variability in the results at each of the temperature levels, the overall trend is unmistakable. The apparent rise in the lethal value of oxygen at 18°C. is, unfortunately, based on the results of only two experiments and obviously a rigid interpretation cannot be given. Two things may be noted, however; first, the temperature was at least 3°C. lower than any temperature experienced by the iao in its natural environment; and secondly, the period of adjustment to this temperature was probably too short. Both factors could contribute to the high lethal oxygen value obtained.

Table 10.--Lethal values of oxygen for individual iao at different temperatures

Experiment No.	Temperature	Lethal O_2	Length of fish	Weight of fish	Duration of experiment
	°C.	cc./l.	mm.	gm.	
1	30.6	1.13	48	1.25	5hr50min
		1.18	48	1.34	5hr15min
	25.2	0.72	46	1.02	9hr10min
	22.0	0.70	40	0.62	11hr05min
2	31.0	0.94	49	1.52	3hr30min
		1.16	51	1.70	3hr
	26.1	0.74	47	1.27	3hr25min
	22.8	0.74	48	1.26	3hr20min
3	31.3	1.02	46	1.20	3hr15min
		1.12	49	1.46	2hr45min
	25.9	0.82	49	1.59	6hr40min
		0.84	50	1.62	4hr25min
	21.8	1.03	45	1.31	5hr30min
		0.58	50	1.70	6hr
4	30.5	1.21	46	1.20	1hr50min
		1.20	51	1.66	2hr13min
	25.8	0.91	54	1.99	2hr35min
	21.1	0.69	52	1.59	6hr40min
5	30.6	1.16	48	1.25	2hr45min
	27.6	0.97	54	2.17	2hr10min
		1.21	45	1.12	3hr
	25.2	0.91	50	1.41	3hr
		0.80	51	1.46	2hr40min
	20.8	0.78	51	1.54	5hr10min
6	27.8	0.96	56	1.79	2hr45min
		1.04	50	1.54	3hr05min
		0.99	50	1.50	4hr
	21.8	0.72	56	2.47	3hr05min
7	21.2	0.78	55	2.03	3hr25min
		0.74	52	1.85	3hr20min
	*18.0	1.56	41	0.75	5hr30min
		1.70	49	1.00	4hr10min

*Acclimated for 24 hours at 18°-19°C.

Discussion

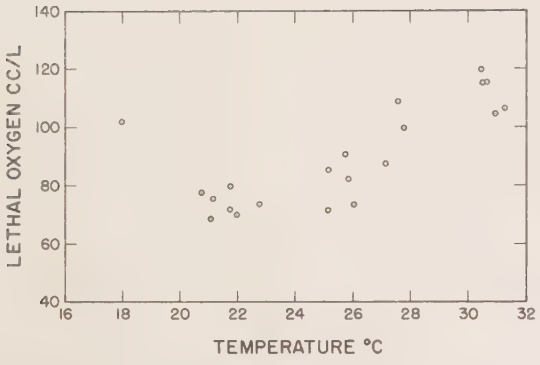

Figure 12.--Lethal values of oxygen at different temperatures. Points represent the average of two trials at each temperature.

Lethal values of oxygen for individual iao ranged from 0.50 to 1.58 cc./l. (combined summer and winter data). There was less variability in the winter data, as evidenced by the smaller standard deviation (0.18 cc./l. as compared with 0.22 cc./l. for the summer data). Some of the variability in any one season's experiments was probably caused by differences in the temperature of the experiments and possibly in the size of the fish used, although the influence of the latter is doubtful. The range of temperatures in the summer experiments was greater than in the winter tests, which could serve to increase the variability of the summer data. Furthermore, in certain instances where fish of exactly the same weight were used, the lethal values of oxygen were identical, but the duration of the respective experiments differed markedly, indicating a marked difference in metabolic rate between the fishes concerned.

Comparing the results on nehu with those of Suehiro (1951), who also worked with individual fish, it was apparent that the variation in his data was greater than that found in the present paper. Standard deviations calculated by the author from Suehiro's data were 0.54 for sardines and 0.44 for anchovies, as compared with 0.35 for the nehu. It is noteworthy that the mean lethal value of oxygen for nehu (2.02 cc./l.) agrees quite well with the mean values found by Suehiro for Japanese bait fishes (2.04 cc./l. for sardines and 1.80 cc./l. for anchovies).

Experiments using many fish gave a lower average lethal value of oxygen than tests on individuals although the range was about the same. The lethal values fell, for the most part, within the range from 0.55-0.85 cc./l. The reason for the lower average value is obscure, but the fish certainly appeared more quiescent when tested in groups rather than individually. Most species of trout appear, like the iao, to have a relatively narrow asphyxial range of oxygen concentrations (Gardner and King 1922, King 1943). Wilding's (1939) curves for three species of freshwater fish (yellow perch, steel-colored shiner, and blunt-nosed minnow) are similar in shape to the one for iao (fig. 10), but the total range in lethal values of oxygen is much greater in his case. It is noteworthy that Wilding withdrew oxygen samples when one or more of the fish showed symptoms of asphyxiation, as evidenced by loss of balance, irregular motion, etc. Complete asphyxiation is perhaps a better criterion to use in controlled laboratory experiments. Wells (1913), for example, states that the "turning-over time" for fish varies considerably within a species, some fish maintaining their equilibrium up to the point of complete asphyxiation. Observations by the writer also indicate a highly variable physical response by iao to lack of oxygen. Comparison with the results of other workers must, of course, be made with caution since the techniques employed are far from standardized. Furthermore, much of the material reported in the literature pertains to field observations on behavior of fish at low oxygen concentrations rather than laboratory experiments.

With the data available, no pronounced relationship between the size of the fish and its resistance to lack of oxygen could be demonstrated. This is somewhat contrary to the results of Keys (1931), who found that larger Fundulus were more resistant to asphyxiation and states that this was due to the smaller oxygen demand per unit weight of the larger fish. Moore (1942), working in the field with several freshwater species, also observed that larger fishes survived critical oxygen concentrations longer than smaller individuals of the same species. On the other hand, Wells (1913) found just the opposite for trout, small fish showing a greater resistance per unit weight than large ones. Suehiro's (1951) data on individual fish present a better comparison with the results reported in this paper. His weights, for both sardines and anchovies, when plotted against the lethal value of oxygen, are even more randomly scattered. It would seem, for iao at

any rate, that regardless of a probable higher metabolic rate in the smaller fish, they are not markedly hindered in their ability to extract oxygen from water low in oxygen content.

A small, but nonetheless statistically significant, difference was found between the mean summer lethal value of oxygen (1.0 cc./l., average temperature 25.6°C.) and the mean winter value (0.96 cc./l., average temperature 21.9°C.). This difference is of about the expected magnitude according to the relationship of temperature to the lethal value of oxygen (fig. 12). In the absence of the appropriate data, however, it is difficult to determine the possible influence of purely seasonal differences in the behavior of the fish.

A few investigators (Gardner and King 1922; Moore 1942; Fry et al. 1947; Graham 1949) have studied the effects of temperature on lethal values of oxygen in fish and found, as in iao, that the lethal level of oxygen increases as the temperature increases. The curve of temperature versus lethal values of oxygen (fig. 12) shows a somewhat steeper rise from 26° to 31°C., roughly corresponding to the pattern shown in the relationship of metabolic rate to temperature (fig. 4). Thus, with a greatly increased metabolic rate at the higher temperatures, the fish is less able to cope with low values of dissolved oxygen. This observation may be of some importance to the live-bait fishery. As mentioned earlier, the fishery is conducted primarily in the summer months, when temperatures often rise above 26°C., particularly in calm anchorages. At the higher temperatures, even a small increase in temperature may bring about a marked decrease in the resistance of the fish to oxygen deficiency.

The lethal values of oxygen reported here from laboratory experiments should be checked by more extensive measurements taken directly from the live-wells of the fishing boats. Such tests might possibly give lethal values intermediate between the average for iao (about 1.0 cc./l.) and that for nehu (about 2.0 cc./l.), since many bait loads are "mixed", containing both nehu and iao.

SUMMARY AND CONCLUSIONS

1. Confinement of tuna baitfish in small live-wells pointed to the desirability of a study of the oxygen requirements of the fish. Experiments were designed to determine lethal levels of oxygen and the rate of oxygen consumption under various conditions. In the oxygen consumption experiments special emphasis was laid on the effects of crowding, flow-rates, and the oxygen concentration of the water.

2. A flowing-water method was used to measure oxygen consumption, dissolved oxygen being determined by the unmodified Winkler procedure. For tests of lethal values of oxygen, where the fish were asphyxiated in sealed jars, the permanganate modification of Winkler's technique was employed.

3. The results are summarized below:

   a. From serial observations, it was evident that oxygen consumption in the iao showed considerable hour-to-hour fluctuation even when there were no outward signs of increased or decreased activity on the part of the fish. No rhythmic cycle in metabolic rate could be demonstrated, at least during the first 24 hours in the container.

   b. Oxygen consumption increased with increase in flow-rate, the effect being perhaps more pronounced in the summer months. In addition, oxygen consumption per unit weight was significantly lower in the tests with large numbers of fish. Possible reasons for the latter finding were discussed.

   c. The "critical oxygen level", i.e., the point at which the rate of oxygen consumption became dependent upon the oxygen concentration of the water, could not be precisely delimited but lay somewhere between 1.5 and 2.5 cc./l. Below this level, both breathing rate and oxygen consumption steadily decreased.

d. Individual iao showed marked differences in resistance to oxygen deficiency. Lethal values of oxygen ranged from 0.50 to 1.58 cc./l., the mean of the summer tests being 1.10 cc./l. and of the winter tests, 0.96 cc./l. A similar range in lethal values was obtained from the experiments where several fish were asphyxiated at once, but almost all the values fell within the narrow interval from 0.55 to 0.85 cc./l., depressing the average to 0.69 cc./l.

e. Over the temperature interval from 19° to 29°C., the rate of oxygen consumption increased slowly at first and then more rapidly at the higher temperatures. Lethal values of oxygen, measured over the same range, also showed a greater increase between 26° and 31°C.

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